## REMARKS

Claims 1-6 are pending. Due to a restriction requirement, claims 4-6 are withdrawn. Claim 2 is rejected under 35 U.S.C. § 102. Claim 3 is rejected under 35 U.S.C. § 103(a). Claims 1 and 3 are rejected under 35 U.S.C. § 112, first paragraph. Each of these rejections is addressed below.

### Claim amendments

Applicants have canceled withdrawn claims 4-6. In addition, without agreeing with the Office and solely to expedite prosecution, Applicants have canceled claim 2. Applicants reserve the right to pursue the canceled claims in this or a continuing application. In view of the cancellation of claim 2, the dependency of claim 3 has been amended.

### Rejection under 35 U.S.C. § 102(b)

Claim 2 is rejected, under 35 U.S.C. § 102(b), as anticipated by Myers et al. (*PNAS* 94:9052-9057, 1997; hereafter "Myers"). As claim 2 has been canceled, this rejection is moot.

### Rejection under 35 U.S.C. § 103(a)

The Office rejects claim 3 under 35 U.S.C. § 103(a) over Myers as applied to

claim 2 under 35 U.S.C. § 102(b) and Maehama et al. (*J. Biol. Chem. 237*:13375-13378, 1998, hereafter "Maehama").

As noted above, claim 2 has been canceled and claim 3, as amended, depends solely from claim 1. Consequently, claim 3 is now directed to a method of diagnosing an impaired glucose tolerance condition, obesity, or propensity thereto in a patient. This method involves analyzing the level of PTEN expression or activity in a sample isolated from the patient. Neither Myers nor Maehama teaches or suggests such a method, and Applicants therefore submit that claim 3 is free of this basis for rejection.

Rejections under 35 U.S.C. § 112, first paragraph

Claims 1 and 3 are also rejected under 35 U.S.C. § 112, first paragraph, based on an asserted lack of enablement. This rejection is respectfully traversed.

*The References Cited by the Office Do Not Support a Lack of Enablement*

The Office cites several references in support of the assertion that "development of impaired glucose tolerance and obesity is multi-factorial and complex," and uses this as a basis for the enablement rejection. In particular, the Office cites Hirosumi et al. (*Nature* 420:333-336, 2002; hereafter "Hirosumi"), Shulman (*Clin. Invest.* 106:171-176, 2000; hereafter "Shulman"), Lönnqvist et al. (*Nat. Med.* 1:950-953, 1995; hereafter "Lönnqvist"), Fontaine (*JAMA* 289:187-193, 2003; hereafter "Fontaine"), Kahn and Flier

(*J. Clin. Invest.* 106:473-481, 2000; hereafter "Kahn"), and Ogg and Ruvkun (Mol. Cell 2:887-893, 1998; hereafter "Ogg"). These references are unavailing, however, as they do not support a lack of enablement for Applicants' presently claimed diagnostic method.

Taking each reference in turn, Hirosumi describes a role for c-Jun amino-terminal kinases in obesity and insulin resistance. Shulman provides an overview of the cellular mechanisms that contribute to insulin resistance. Lönnqvist deals with understanding obesity and developing routes for its pharmacological treatment; in particular, Lönnqvist describes the overexpression of the obese (ob) gene in adipose tissue of obese human subjects. Fontaine describes differences in life expectancy observed for obese versus normal human subjects. Hirosumi, Shulman, Lönnqvist, and Fontaine uniformly fail to address the role of PTEN in mammalian glucose metabolism and fail to address the possible use of PTEN level or activity in diagnosing an impaired glucose tolerance condition or obesity.

Kahn provides a review of the role of insulin resistance in diabetes and the relationship of obesity to insulin resistance and type 2 diabetes. The Office, citing Kahn, asserts (page 9):

> The applicant fails to consider the complexities involved in the mammalian insulin signal transduction pathway especially in context with impaired glucose tolerance and the development [of] obesity.

Applicants note that the claimed methods are directed to diagnosis of an impaired glucose tolerance condition, obesity, or a propensity thereto. These methods do not require a

complete understanding of the insulin signaling pathway, but rather require that the level of PTEN expression or activity, relative to a control, be indicative of an impaired glucose tolerance condition or obesity. As discussed in greater detail below, Applicants' specification clearly enables this method.

Further, Kahn teaches (page 473, left column):

> Insulin's metabolic effects are mediated by a broad array of tissue-specific actions that involve rapid changes in protein phosphorylation and function, as well as changes in gene expression. The fundamental biologic importance of these actions of insulin is evidenced by the fact that the insulin signaling cascade which initiates these events is largely conserved in evolution from *C. elegans* to humans.

Thus, rather than calling into question Applicants' diagnostic approach, Kahn recognizes that significant parallels exist between *C. elegans* and mammalian insulin signaling. The reference therefore supports Applicants' position.

The Office, citing the final reference by Ogg, asserts (page 8):

> The state of the art a[t] the time of filing was such that it has been unclear whether the PTEN (daf-18) activity is regulated during insulin-like signaling or any other signaling activity, since PTEN lipid phosphatase activity is low in vitro due to a missing modification of the insulin signaling cascade.

Applicants note that the teachings of Ogg are mischaracterized. The section of Ogg relied on by the Office reads (page 891, top of right column):

> One attractive possibility is that the AKT kinases or PDK1 activates DAF-18 as a component in the recovery from an episode of insulin signaling. It may be significant that PTEN lipid phosphatase activity is low in vitro, perhaps due to a missing modification by the insulin signaling cascade.

(emphasis added; citation omitted)

Clearly, Ogg is simply providing a possible reason for why others have observed low PTEN phosphatase activity *in vitro*. Nonetheless, citing this section of Ogg, the Office states (page 8):

> Since the factors that affect PTEN activity in vivo are not well understood it is unclear what would be a representative control sample that can be used to evaluate the claimed PTEN activity.

This concern is misplaced. Applicants' specification establishes a role for PTEN in mammalian glucose homeostasis. Applicants teach (page 117, lines 3-7):

> Reduction in PTEN activity would be expected to potentiate insulin and/or insulin-like growth factor signaling, but an increase in PTEN activity would be expected to cause insulin resistance downstream of the insulin receptor, the type observed in late onset diabetes.

Applicants, like the Kahn reference relied on by the Office, disclose that results regarding the regulation of insulin signaling in *C. elegans* are relevant to mammalian insulin signaling.

> These results further endorse the congruence between the *C. elegans* and mammalian insulin signaling pathways, strongly supporting the contention that new genes identified in the *C. elegans* pathway also act in mammalian insulin signaling. In addition, we have also found that the *C. elegans* PTEN lipid phosphatase homologue, DAF-18, acts upstream of AKT in this signaling pathway. Thus, our molecular genetic analysis maps mammalian PTEN action to the insulin signaling pathway. (page 3, lines 9-15 of the specification)

Further, in the last reply, Applicants pointed to Butler et al. (*Diabetes* 51:1028-1034,

2002; hereafter "Butler") as evidence that, as disclosed in Applicants' specification, PTEN functions in mammalian glucose homeostasis. Here Applicants again note that Butler teaches (in the abstract):

> Systemic administration of PTEN ASO [antisense oligonucleotides] once a week in mice suppressed PTEN mRNA and protein expression in liver and fat by up to 90 and 75% respectively, and normalized blood glucose concentrations in *db/db* and *ob/ob* mice. Inhibition of PTEN expression also dramatically reduced insulin concentrations in *ob/ob* mice, improved the performance of *db/db* mice during insulin tolerance tests, and increased Akt phosphorylation in liver in response to insulin. These results suggest that PTEN plays a significant role in regulating glucose metabolism in vivo by negatively regulating insulin signaling. (emphasis added)

The Office dismissed this evidence in the present Office Action, stating (page 9):

> Applicant's argument that Butler et al. … teaches that PTEN modulates mammalian insulin signaling just as the applicant disclosed has been found unpersuasive because each patent application is examined on its own merits and is considered enabled in view of its own disclosure. The issue is not whether other application[s] support their claims but whether one supports its claims. (emphasis original; citation omitted)

Applicants respectfully point out that, as noted above, the specification clearly teaches that a reduction or an increase in PTEN expression or function can be used in diagnosing an impaired glucose tolerance condition, obesity, or a propensity thereto in a patient, as is presently claimed. Butler simply confirms the role of PTEN in mammalian insulin signaling, and thereby supports Applicants' argument that the specification enables the

present diagnostic claims.

Further, Applicants submit that, in determining whether or not the claims in an application are enabled at the time the application was filed, the Office must consider post-filing art. On this point, the Federal Circuit has held:

> [I]t is true that a later dated publication cannot supplement an insufficient disclosure in a prior dated application to render it enabling. In this case, the later dated publication was not offered as evidence for this purpose. Rather, it was offered as evidence of the level of ordinary skill in the art at the time of the publication and as evidence that the disclosed device would have been operative. *Gould v. Quigg*, 822 F.2d 1074, 3 USPQ2d 1302 (Fed. Cir. 1987).

As in *Gould*, Applicants presented the Butler reference in support of their assertion that the presently claimed diagnostic method is workable and not to supplement their disclosure. Thus, Applicants submit that the Office must consider Butler, and other post-filing art, in deciding whether or not PTEN plays a role in mammalian insulin signaling.

As further evidence of the workability of Applicants' invention, yet another post-filing publication, Stiles et al., *Proc. Natl. Acad. Sci. USA* 101:2082-2087, 2004 (hereafter "Stiles") is submitted herewith. Stiles teaches (in the abstract):

> *Pten* liver-specific deletion causes enhanced liver insulin action with improved systemic glucose tolerance. Thus, deletion of *Pten* in the liver may provide a valuable model that permits the study of metabolic actions of insulin signaling in the liver.

Clearly, as taught in Applicants' specification as filed, and as supported by further post-filing experimental data, PTEN plays a role in mammalian glucose homeostasis and

therefore provides a valuable diagnostic for impaired glucose tolerance conditions and obesity. Nothing in the references cited by the Office refutes this evidence, and this basis for the enablement rejection should be withdrawn.

*Enablement of Diagnostic Methods*

The enablement rejection is also based on the assertion by the Office that Applicants' specification fails to enable one skilled in the art to make and use the presently claimed method. Applicants disagree. The test of enablement is "whether one reasonably skilled in the art could make or use the invention from the disclosures in the patent coupled with the information known in the art without undue experimentation." *Hybritech, Inc. v. Monoclonal Antibodies, Inc.* 802 F.2d. 1318 (Fed. Cir. 1985). The test for undue experimentation is not merely quantitative, since a considerable amount of experimentation is permissible if it is merely routine, or if the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed. Applicants' specification meets this standard.

As noted above, the specification teaches, at page 117, lines 3-7, that a reduction in PTEN activity potentiates insulin and/or insulin-like growth factor signaling, whereas an increase in PTEN activity causes insulin resistance downstream of the insulin receptor. Thus, to diagnose an impaired glucose tolerance condition or obesity, one merely needs to determine if a patient exhibits increased PTEN levels.

Methods for analyzing the level of PTEN activity are described, for example, at page 119, lines 2-5, of the specification. There, Applicants teach that DAF-18/PTEN activity may be identified using an *in vitro* lipid phosphatase assay as described by Maehama. Alternatively, methods for analyzing PTEN expression levels are described, for example, at pages 150-152, where Applicants teach that anti-DAF polypeptide antibodies are useful in immunological assays to detect DAF polypeptide expression in a patient sample; at pages 176 and 177, where Applicants teach that a DAF promoter may be fused to a reporter and used to monitor DAF expression; and at page 195, lines 10-25, where Applicants teach methods of analyzing the expression of DAF-18 nucleic acid and amino acid sequences. These assays each determine PTEN levels and thus Applicants' specification teaches how to make and use the presently claimed diagnostic method.

The Office also rejects the present claims based on the assertion that "it is unclear what would be a representative control sample" for diagnosing an increase in PTEN expression or activity. Clearly, an appropriate control would be one obtained from an individual who does not have an impaired glucose tolerance condition, is not obese, and does not have a propensity for acquiring these conditions. Such controls are referred to in Applicants' specification, for example, at page 126, lines 10-12, where Applicants teach that the appropriate controls for studies of insulin regulation are wild-type control mammals. Choosing an appropriate control is therefore standard in view of Applicants' specification and in the art, and could readily be accomplished by a skilled artisan

carrying out the presently claimed methods.

The Office further asserts (page 10):

> [A]nalyzing any and all kind of PTEN lipid phosphatase activity in any and all tissue sample[s] is not considered routine in the art.

On this point, Applicants' first note that, as pointed out above, the specification teaches a method of determining PTEN lipid phosphatase activity that is standard in the art. This method can be used to carry out the presently claimed method and, therefore, enables this aspect of the claimed method. The enablement standard does not require Applicants to enable any and all PTEN lipid phosphatase assays.

Moreover, one skilled in the art would clearly compare tissues that normally express PTEN. As noted above, analyzing the level of PTEN expression is taught, for example, at pages 150-152 of Applicants' specification. Using this technique, one skilled in the art could readily determine which tissues (such as fat) should be compared. One skilled in the art need not compare PTEN lipid phosphatase activity in any and all tissues; no undue experimentation is required to select a tissue sample.

The Office also notes that "the specification fails to provide a single working example, which establishes that PTEN modulates mammalian insulin signaling." As noted above, the standard for enablement is whether one reasonably skilled in the art could make or use the invention from the disclosures in the patent application coupled with the information known in the art without undue experimentation. Applicants'

specification teaches that PTEN plays a role in mammalian glucose homeostasis, a finding that was confirmed by others after the application was filed. As pointed out above, Applicants' specification also teaches how one skilled in the art can make and use the presently claimed method using nothing more than the disclosure of the specification and standard knowledge in the art. Thus, Applicants submit that undue experimentation is not required to make and use the presently claimed invention and, on this basis, Applicants' specification meets the enablement standard, regardless of whether or not it contains a working example.

*Scope of the Claims*

As a final basis for the enablement rejection, the Office asserts that "it is unclear how one skill[ed] in the art would diagnose [an] impaired tolerance condition or propensity thereto by analyzing PTEN lipid phosphatase activity alone in type-I diabetic patients, wherein the impaired glucose tolerance is the result of loss of insulin secretion." Applicants submit that analyzing the level of PTEN expression or activity by measuring PTEN lipid phosphatase activity expression or action is workable even in the absence of insulin. As is noted above, the assay involves comparing the PTEN lipid phosphatase activity in a sample isolated from a patient with a control sample from an individual who does not have an impaired glucose tolerance condition, obesity, or a propensity thereto.

Applicants' specification, for example, at page 108, lines 15-17, teaches that

"PTEN has lipid phosphatase activity that dephosphorylates position 3 on the inositol ring of $PIP_3$ *in vitro* and decreases the level of the lipid products of PI3K in response to insulin signaling in human 293 cells." As insulin signaling is regulated by PTEN lipid phosphatase activity, in the absence of insulin signaling, i.e., in the absence of insulin, PTEN lipid phosphatase activity would clearly be different than in its presence. Thus, one skilled in the art carrying out the claimed method would observe a difference in PTEN expression or activity between the sample from a patient who does not secrete insulin (e.g., a type I diabetic) and the control. This basis for rejection should also be withdrawn.

In sum, Applicants submit that one skilled in the art can make and use the presently claimed methods without undue experimentation. Applicants' discovery that an increase in PTEN expression or activity relative to a control is indicative of a patient having an impaired glucose tolerance condition, obesity, or a propensity thereto, enables one skilled in the art to carry out the presently claimed diagnostic methods using nothing more than the teachings of Applicants' specification and standard methods. For all the above reasons, the enablement rejection under 35 U.S.C. § 112, first paragraph, should be withdrawn.

## CONCLUSION

Applicants submit that this case is in condition for allowance, and such action is respectfully requested. Enclosed are a petition for extending the period for reply for three months, to and including, October 19, 2004, and a check for the required extension fee. If there are any other charges, or any credits, please apply them to Deposit Account No. 03-2095.

Respectfully submitted,

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